



Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 4A

SEQ ID No. 3. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 171500, PCR type 1, with translation. The putative secretory signal cleavage site ( $\Delta$ ) and site of cleavage to form the two mature SLPs ( $\diamond$ ) are indicated.

```
1 ATGAATAAGAAAATATAGCAATAGCTATGTCAGGTTTAAACAGTTTCTAGCTTCGGCTGCA 60
  -----+-----+-----+-----+-----+
1 M N K K N I A I A M S G L T V L A S A A 20

61 CCTGTATTTGCAGATGATACAAAAGTTGAACTGGTGATCAAGGATATACAGTGGTACAA 120
  -----+-----+-----+-----+-----+
21 P V F A D D T K V E T G D Q G Y T V V Q 40
    Δ

121 AGCAAGTATAAGAAAGCTGTTGAACAATTACAAAAGGAATATTAGATGGAAGTATAACA 180
  -----+-----+-----+-----+-----+
41 S K Y K K A V E Q L Q K G I L D G S I T 60

181 GAAATTAAAGTTTCTTTGAGGGAACCTTAGCATCTACTATAAAAGTAGGTTCTGAGCTT 240
  -----+-----+-----+-----+-----+
61 E I K V F F E G T L A S T I K V G S E L 80

241 AATGCAGCAGATGCAAGTAAATTATTGTTTACACAAGTAGATAATAAACTAGATAATTTA 300
  -----+-----+-----+-----+-----+
81 N A A D A S K L L F T Q V D N K L D N L 100

301 GGTGATGGAGATTATGTAGATTCTTAATAACTTCTCCAGGTCAAGGGGATAAAATAACT 360
  -----+-----+-----+-----+-----+
101 G D G D Y V D F L I T S P G Q G D K I T 120

361 ACAAGTAAACTTGTTCATTGAAAGATTTAACAGGTGCTTCAGCAGATGCTATAATTGCT 420
  -----+-----+-----+-----+-----+
121 T S K L V A L K D L T G A S A D A I I A 140

421 GGAACATCTTCAGCAGATGGTGTGTTACAAATACTGGAGCTGCTAGTGGTTCTACTGAG 480
  -----+-----+-----+-----+-----+
141 G T S S A D G V V T N T G A A S G S T E 160

481 ACAAATTCAGCAGGAACAAAACCTTGCAATGTCAGCTATTTTTGACACAGCATATACAGAT 540
  -----+-----+-----+-----+-----+
161 T N S A G T K L A M S A I F D T A Y T D 180

541 TCATCTGAAACTGCGGTTAAGATTACTATAAAAGCAGATATGAATGATACTAAATTGGT 600
  -----+-----+-----+-----+-----+
181 S S E T A V K I T I K A D M N D T K F G 200

601 AAAGCAGGTGAGACAACTTATTCAACTGGGCTTACATTGAAGATGGGTCTACAGAAAAA 660
  -----+-----+-----+-----+-----+
201 K A G E T T Y S T G L T F E D G S T E K 220
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 4B

661 ATTGTTAAATTAGGGGACAGTGATATTATAGATATAACTAAAGCTCTTAACTTACTGTT 720  
-----+-----+-----+-----+-----+-----+-----+  
221 I V K L G D S D I I D I T K A L K L T V 240

721 GTTCCTGGAAGTAAAGCAACTGTTAAGTTTGCTGAAAAACACCAAGTGCCAGTGTTCAA 780  
-----+-----+-----+-----+-----+-----+-----+  
241 V P G S K A T V K F A E K T P S A S V Q 260

781 CCAGTAATAACAAAGCTTAGAATAATAAATGCTAAAGAAGAAACAATAGATATTGACGCT 840  
-----+-----+-----+-----+-----+-----+-----+  
261 P V I T K L R I I N A K E E T I D I D A 280

841 AGTTCTAGTAAACAGCACAGATTAGCTAAAAATATGTATTTAATAAACTGATTTA 900  
-----+-----+-----+-----+-----+-----+-----+  
281 S S S K T A Q D L A K K Y V F N K T D L 300

901 AATACTCTTTATAAAGTATTAAATGGAGATGAAGCAGATACTAATGGATTAATAGAAGAA 960  
-----+-----+-----+-----+-----+-----+-----+  
301 N T L Y K V L N G D E A D T N G L I E E 320

961 GTTAGTGGAAATATCAAGTAGTTCTTTATCCAGAAGGAAAAGAGTTACAACCTAAGAGT 1020  
-----+-----+-----+-----+-----+-----+-----+  
321 V S G K Y Q V V L Y P E G K R V T T K S 340

1021 GCTGCAAGGCTTCAATTGCTGATGAAAATTCACCAAGTTAAATTAAGTCAAGT 1080  
-----+-----+-----+-----+-----+-----+-----+  
341 A A K A S I A D E N S P V K L T L K S D 360

1081 AAGAAGAAAGACTTAAAGATTATGTGGATGATTTAAGAACATATAATAATGGATATTCA 1140  
-----+-----+-----+-----+-----+-----+-----+  
361 K K K D L K D Y V D D L R T Y N N G Y S 380

1141 AATGCTATAGAAGTAGCAGGAGAAGATAGAATAGAACTGCAATAGCATTAGTCAAAAA 1200  
-----+-----+-----+-----+-----+-----+-----+  
381 N A I E V A G E D R I E T A I A L S Q K 400

1201 TATTATAACTCTGATGATGAAAATGCTATATTTAGAGATTCAGTTGATAATGTAGTATTG 1260  
-----+-----+-----+-----+-----+-----+-----+  
401 Y Y N S D D E N A I F R D S V D N V V L 420

1261 GTTGGAGGAAATGCAATAGTTGATGGACTTGCTAGCTTCTCCTTTAGCTTCTGAAAAGAAA 1320  
-----+-----+-----+-----+-----+-----+-----+  
421 V G G N A I V D G L V A S P L A S E K K 440

1321 GCTCCTTTATTATTAACCTTCAAAAGATAAATTAGATTCAAGCGTAAAGCTGAAATAAAG 1380  
-----+-----+-----+-----+-----+-----+-----+  
441 A P L L L T S K D K L D S S V K A E I K 460

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 4C

1381	AGAGTTATGAATATAAAGAGTACAACAGGTATAAATACTTCAAAGAAAGTTTATTAGCT	1440
461	R V M N I K S T T G I N T S K K V Y L A	480
1441	GGTGGAGTTAATTCTATATCTAAAGAAGTAGAAAATGAATTAAGATATGGGACTTAAA	1500
481	G G V N S I S K E V E N E L K D M G L K	500
1501	GTTACAAGATTAGCAGGAGATGATAGATATGAACTTCTCTAAAAATAGCTGATGAAGTA	1560
501	V T R L A G D D R Y E T S L K I A D E V	520
1561	GGTCTTGATAATGATAAAGCATTGTAGTTGGAGGAACAGGATTAGCAGATGCCATGAGT	1620
521	G L D N D K A F V V G G T G L A D A M S	540
1621	ATAGCTCCAGTTGCATCTCAATTAAGAAATGCTAATGGTAAAATGGATTAGCTGATGGT	1680
541	I A P V A S Q L R N A N G K M D L A D G	560
1681	GATGCTACACCAATAGTAGTTGTAGATGGAAAAGCTAAACTATAAATGATGATGTAAAA	1740
561	D A T P I V V V D G K A K T I N D D V K	580
1741	GATTTCTTAGATGATTCACAAGTTGATATAATAGGTGGAGAAAACAGTGTATCTAAAGAT	1800
581	D F L D D S Q V D I I G G E N S V S K D	600
1801	GTTGAAAATGCAATAGATGATGCTACAGGTAAATCTCCAGATAGATATAGTGGAGATGAT	1860
601	V E N A I D D A T G K S P D R Y S G D D	620
1861	AGACAAGCAACTAATGCAAAAGTTATAAAGAATCTTCTTATTATCAAGATAACTTAAAT	1920
621	R Q A T N A K V I K E S S Y Y Q D N L N	640
1921	AATGATAAAAAAGTAGTTAATTTCTTTGTAGCTAAAGATGGTTCTACTAAAGAAGATCAA	1980
641	N D K K V V N F F V A K D G S T K E D Q	660
1981	TTAGTTGATGCTTTAGCAGCAGCTCCAGTTGCAGCAAACTTTGGTGTAACCTCTTAATTCT	2040
661	L V D A L A A A P V A A N F G V T L N S	680
2041	GATGGTAAGCCAGTAGATAAAGATGGTAAAGLATTAACTGGTTCTGATAATGATAAAAAAT	2100
681	D G K P V D K D G K V L T G S D N D K N	700
2101	AAATTAGTATCTCCAGCACCTATAGTATTAGCTACTGATTCTTTATCTTCAGATCAAGT	2160
701	K L V S P A P I V L A T D S L S S D Q S	720

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 4D

```
2161  GTATCTATAAGTAaAGTTCTTGATAAAGATAATGGAGAAAACCTTAGTTCAAGTTGGTAAA  2220
-----+-----+-----+-----+-----+
721  V S I S K V L D K D N G E N L V Q V G K  740

2221  GGTATAGCTACTTCAGTTATAAACAAAATGAAAGATTTATTAGATATG  2268
-----+-----+-----+-----+
741  G I A T S V I N K M K D L L D M  756
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 5A

SEQ ID No. 4. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 172450, PCR type 5, with translation. The putative secretory signal cleavage site ( $\Delta$ ) is indicated, and an approximation of the and site of cleavage to form the two mature SLPs ( $\diamond$ ) is also indicated.

```
1 ATGAAAAAAGAAATTTAGCAATGGCTATGGCAGCTGTTACTGTAGTAGGTTCTGCTGCT 60
-----+-----+-----+-----+
1 M K K R N L A M A M A A V T V V G S A A 20

61 CCAGTTTTTGCAGCAGCTTCAGATGTAATATCACTACAAGATGGTACAAATGATAAGTAT 120
-----+-----+-----+-----+
21 P V F A A A S D V I S L Q D G T N D K Y 40
      Δ

121 ACAGTATCAAATACTAAAGCTAGTGACTTAGTAAAGGATATTTTAGCAGCACAAAACCTTA 180
-----+-----+-----+-----+
41 T V S N T K A S D L V K D I L A A Q N L 60

181 ACAACAGGTGCAGTTATTTTGAACAAAGATACAAAAGTTACTTTCTATGATGCAATGAG 240
-----+-----+-----+-----+
61 T T G A V I L N K D T K V T F Y D A N E 80

241 AAAGATTCTTCAACTCCAAGTGGAGATAAAAAAGTTTATTCAGAACAACCTTTAACTACA 300
-----+-----+-----+-----+
81 K D S S T P T G D K K V Y S E Q T L T T 100

301 GCTAATGGAAATGAAGATTATGTAAAGACAACCTTTAAAAAATTTAGATGCAGGAGAATAT 360
-----+-----+-----+-----+
101 A N G N E D Y V K T T L K N L D A G E Y 120

361 GCTATTATAGATTTAACTTATAATGCTAAACTGTTGAAATTAAGTAGTAGCAGCT 420
-----+-----+-----+-----+
121 A I I D L T Y N N A K T V E I K V V A A 140

421 AGTGAAAAACAGTAGTTGTATCTAGTGATGCGAAAAATAGTGCAAAAGATATAGCTGAA 480
-----+-----+-----+-----+
141 S E K T V V V S S D A K N S A K D I A E 160

481 AAATATGTGTTTGAAGACAAAGACTTAGAAAAATGCACTAAAAACTATAAATGCCTCAGAT 540
-----+-----+-----+-----+
161 K Y V F E D K D L E N A L K T I N A S D 180

541 TTCAGTAAACTGATAGTTACTATCAAGTAGTTCTTTATCCAAAAGGAAAGAGATTACAA 600
-----+-----+-----+-----+
181 F S K T D S Y Y Q V V L Y P K G K R L Q 200

601 GGTTCCTCAACTTATAGAGCTACAAATTATAATGAAGGAAGTGCATATGGTAATACACCA 660
-----+-----+-----+-----+
201 G F S T Y R A T N Y N E G T A Y G N T P 220
      ♦
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 5B

661	GTAATATTAACTCTAAAATCTACTAGTAAGAGTAATTTAAAGACTGCAGTAGAAGAGTTA	720
221	V I L T L K S T S K S N L K T A V E E L	240
721	CAAAAATTGAATGCTAGTTATTCTAATACTACAACCTTTAGCTGGTGATGACAGAATACAA	780
241	Q K L N A S Y S N T T T L A G D D R I Q	260
781	ACAGCTATAGAGATAAGTAAGAATATTACAATAATGATGGCGAGAAATCAGATCATTCA	840
261	T A I E I S K E Y Y N N D G E K S D H S	280
841	GCTGATGTTAAAGAGAATGTTAAAAATGTTGTATTAGTAGGTGCAAATGCACTAGTAGAT	900
281	A D V K E N V K N V V L V G A N A L V D	300
901	GGATTAGTTGCGGCTCCTTTAGCAGCAGAAAAAGATGCTCCACTATTATTAACCTCAAAA	960
301	G L V A A P L A A E K D A P L L L T S K	320
961	GATAAATTAGATTCGTCAGTAAAATCTGAAATAAAGAGAGTTTtagactTAAAACTTCA	1020
321	D K L D S S V K S E I K R V L D L K T S	340
1021	ACAGAAGTAACAGGAAAAACAGTTTATATAGCTGGTGGAGTTAATAGTGTATCTAAAGAA	1080
341	T E V T G K T V Y I A G G V N S V S K E	360
1081	GTTGTAACAGAATTAGAATCAATGGGATTAAAAGTTGAAAGATTCTCAGGTGATGATAGA	1140
361	V V T E L E S M G L K V E R F S G D D R	380
1141	TATGAAACTTCTTTAAAAATAGCAGGTGAAATAGGCTTAGATAATGATAAGGCTTATGTA	1200
381	Y E T S L K I A G E I G L D N D K A Y V	400
1201	GTTGGTGGAACAGGATTAGCAGATGCCATGAGTATAGCTTCAGTTGCTTCTACTAAATTA	1260
401	V G G T G L A D A M S I A S V A S T K L	420
1261	GATGTAATGGTGTGTGTAGATAGAACAAATGGACATGCTACTCCAATAGTTGTTGTAGAT	1320
421	D G N G V V D R T N G H A T P I V V V D	440
1321	GGAAAAGCTGATAAAATATCTGATGACTTAGATAGTTTCTTAGGAAGCGCTGATGTAGAT	1380
441	G K A D K I S D D L D S F L G S A D V D	460
1381	ATAATAGGTGGATTGCAAGTGTATCTGAAAAGATGGAAGAAGCTATATCAGATGCTACT	1440
461	I I G G F A S V S E K M E E A I S D A T	480

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 5C

```
1441 GGTAAAGGCGTTACAAGAGTTAAAGGCGACGATAGACAAGACACTAACTCTGAAGTTATA 1500
-----+-----+-----+-----+-----+
481 G K G V T R V K G D D R Q D T N S E V I 500

1501 AAAACATATTATGCTAATGATACTGAAATAGCTAAAGCTGCAGTTTATAGATAAAGATTCA 1560
-----+-----+-----+-----+-----+
501 K T Y Y A N D T E I A K A A V L D K D S 520

1561 GGTGCTTCAAGTAGTGATGCAGGAGTATTTAATTTCTATGTAGCTAAAGATGGATCTACA 1620
-----+-----+-----+-----+-----+
521 G A S S S D A G V F N F Y V A K D G S T 540

1621 AAAGAAGATCAATTAGTTGATGCATTAGCAGTAGGAGCTGTTGCTGGATATAAACTTGCT 1680
-----+-----+-----+-----+-----+
541 K E D Q L V D A L A V G A V A G Y K L A 560

1681 CCAGTTGTATTAGCTACTGATTCTTTATCTTCTGATCAATCGGTTGCTATAAGCAAAGTT 1740
-----+-----+-----+-----+-----+
561 P V V L A T D S L S S D Q S V A I S K V 580

1741 GTAGGAGAAAAATATTCTAAAGATTTAACACAAGTTGGTCAAGGAATAGCTAATTCAGTT 1800
-----+-----+-----+-----+-----+
581 V G E K Y S K D L T Q V G Q G I A N S V 600

1801 ATAAACAAAATGAAAGATTTATTAGATATG 1830
-----+-----+
601 I N K M K D L L D M 610
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 6A

SEQ ID No. 5. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 170324, PCR type 12, with translation. The putative secretory signal cleavage site ( $\Delta$ ) and site of cleavage to form the two mature SLPs ( $\diamond$ ) are indicated.

```
1 ATGAATAAGAAAAATATAGCAATAGCTATGTCAGGTTTACAGTTTCTAGCTTCGGCTGCT 60
-----+-----+-----+-----+-----+
1 M N K K N I A I A M S G L T V L A S A A 20

61 CCTGTTTTTGCTGCACTACTGGAACACAAGGTTATACTGTAGTTAAAAACGACTGGAAA 120
-----+-----+-----+-----+-----+
21 P V F A A T T G T Q G Y T V V K N D W K 40
      Δ

121 AAAGCAGTAAACAATTACAAGATGGACTAAAAGATAATAGTATAGGAAAGATAACTGTA 180
-----+-----+-----+-----+-----+
41 K A V K Q L Q D G L K D N S I G K I T V 60

181 TCTTTTAATGATGGGGTTGTGGGTGAAGTAGCTCCTAAAAGTGCTAATAAGAAACGGGAC 240
-----+-----+-----+-----+-----+
61 S F N D G V V G E V A P K S A N K K A D 80

241 AGAGATGCTGCAGCTGAGAAGTTATATAATCTTGTTAACTCAATTAGATAAATTAGGT 300
-----+-----+-----+-----+-----+
81 R D A A A E K L Y N L V N T Q L D K L G 100

301 GATGGAGATTATGTTGATTTTTCTGTAGATTATAATTTAGAAAACAAAATAATAACTAAT 360
-----+-----+-----+-----+-----+
101 D G D Y V D F S V D Y N L E N K I I T N 120

361 CAAGCAGATGCAGAAGCAATTGTTACAAAGTTAAATTCACCTTAATGAGAAAACCTTTATT 420
-----+-----+-----+-----+-----+
121 Q A D A E A I V T K L N S L N E K T L I 140

421 GATATAGCAACTAAAGATACTTTTGAATGGTTAGTAAACACAAGATAGTGAAGGTAAA 480
-----+-----+-----+-----+-----+
141 D I A T K D T F G M V S K T Q D S E G K 160

481 AATGTTGCTGCAACAAAGGCACTTAAAGTTAAAGATGTTGCTACATTTGTTTGAAGTCT 540
-----+-----+-----+-----+-----+
161 N V A A T K A L K V K D V A T F G L K S 180

541 GGTGGAAGCGAAGATACTGGATATGTTGTTGAAATGAAAGCAGGAGCTGTAGAGGATAAG 600
-----+-----+-----+-----+-----+
181 G G S E D T G Y V V E M K A G A V E D K 200

601 TATGGTAAAGTTGGAGATAGTACGGCAGGTATTGCAATAAATCTTCCTAGTACTGGACTT 660
-----+-----+-----+-----+-----+
201 Y G K V G D S T A G I A I N L P S T G L 220
```



Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 6B

```
661 GAATATGCAGGTAAAGGAACAACAATTGATTTTAATAAAACTTTAAAAGTTGATGTAACA 720
-----+-----+-----+-----+-----+
221 E Y A G K G T T I D F N K T L K V D V T 240

721 GGTGGTTCAACACCTAGTGCTGTAGCTGTAAGTGGTTTTGTAACATAAGATGATACTGAT 780
-----+-----+-----+-----+-----+
241 G G S T P S A V A V S G F V T K D D T D 260

781 TTAGCAAAATCAGGTACTATAAATGTAAGAGTTATAAATGCAAAAGAAGAATCAATTGAT 840
-----+-----+-----+-----+-----+
261 L A K S G T I N V R V I N A K E E S I D 280

841 ATAGATGCAAGCTCATATACATCAGCTGAAAATTTAGCTAAAAGATATGTATTTGATCCA 900
-----+-----+-----+-----+-----+
281 I D A S S Y T S A E N L A K R Y V F D P 300

901 GATGAAATTTCTGAAGCATATAAGGCAATAGTAGCATTACAAAATGATGGTATAGAGTCT 960
-----+-----+-----+-----+-----+
301 D E I S E A Y K A I V A L Q N D G I E S 320

961 AACTTAGTTCAGTTAGTTAATGGAAAATATCAAGTGATTTTTTATCCAGAAGGTAAAAGA 1020
-----+-----+-----+-----+-----+
321 N L V Q L V N G K Y Q V I F Y P E G K R 340

1021 TTAGAAACTAAATCAGCAAATGATACAATAGCTAGTCAAGATACACCAGCTAAAGTAGTT 1080
-----+-----+-----+-----+-----+
341 L E T K S A N D T I A S Q D T P A K V V 360

1081 ATAAAAGCTAATAAATTAAAAGATTTAAAAGATTATGTAGATGATTTAAAACATATAAT 1140
-----+-----+-----+-----+-----+
361 I K A N K L K D L K D Y V D D L K T Y N 380

1141 AATACTTATTCAAATGTTGTAACAGTAGCAGGAGAAGATAGAATAGAACTGCTATAGAA 1200
-----+-----+-----+-----+-----+
381 N T Y S N V V T V A G E D R I E T A I E 400

1201 TTAAGTAGTAAATATTATAATTCTGATGATAAAAATGCAATAACTGATAAAGCAGTTAAT 1260
-----+-----+-----+-----+-----+
401 L S S K Y Y N S D D K N A I T D K A V N 420

1261 GATATAGTATTAGTTGGATCTACATCTATAGTTGATGGTCTTGTTCATCACCATTAGCT 1320
-----+-----+-----+-----+-----+
421 D I V L V G S T S I V D G L V A S P L A 440

1321 TCAGAAAAACAGCTCCATTATTATTAACCTCAAAAGATAAATTAGATTCATCAGTAAAA 1380
-----+-----+-----+-----+-----+
441 S E K T A P L L L T S K D K L D S S V K 460
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 6C

1381	TCTGAAATAAAGAGAGTTATGAACTTAAAGAGTGACACTGGTATAAATACTTCTAAAAAA	1440
461	S E I K R V M N L K S D T G I N T S K K	480
1441	GTATTTAGCTGGTGGAGTTAATTCTATATCTAAAGATGTAGAAAATGAATTGAAAAAC	1500
481	V Y L A G G V N S I S K D V E N E L K N	500
1501	ATGGGTCTTAAAGTTACTAGATTATCAGGAGAAGACAGATACGAAACTTCTTTAGCAATA	1560
501	M G L K V T R L S G E D R Y E T S L A I	520
1561	GCTGATGAAATAGGTCTTGATAATGATAAAGCATTGTAGTTGGTGGTACTGGATTAGCA	1620
521	A D E I G L D N D K A F V V G G T G L A	540
1621	GATGCTATGAGTATAGCTCCAGTTGCTTCTCAACTTAAAGATGGAGATGCTACTCCAATA	1680
541	D A M S I A P V A S Q L K D G D A T P I	560
1681	GTAGTTGTAGATGGAAAAGCAAAGAATAAGTGATGATGCTAAGAGTTTCTTAGGAAGT	1740
561	V V V D G K A K E I S D D A K S F L G T	580
1741	TCTGATGTTGATATAATAGGTGGAAAAATAGCGTATCTAAAGAGATTGAAGAGTCAATA	1800
581	S D V D I I G G K N S V S K E I E E S I	600
1801	GATAGTGCAACTGGAAAACTCCAGATAGAATAAGTGGAGATGATAGACAAGCAACTAAT	1860
601	D S A T G K T P D R I S G D D R Q A T N	620
1861	GCTGAAGTTTTTAAAGAAGATGATTATTTACAGATGGTGAAGTTGTGAATTACTTTGTT	1920
621	A E V L K E D D Y F T D G E V V N Y F V	640
1921	GCAAAAGATGGTTCTACTAAAGAAGATCAATTAGTAGATGCCTTAGCAGCAGCACCAATA	1980
641	A K D G S T K E D Q L V D A L A A A P I	660
1981	GCAGGTAGATTTAAGGAGTCTCCAGCTCCAATCATACTAGCTACTGATACTTTATCTTCT	2040
661	A G R F K E S P A P I I L A T D T L S S	680
2041	GACCAAATGTAGCTGTAAGTAAAGCAGTTCCTAAAGATGGTGGAACTAACTTAGTTCAA	2100
681	D Q N V A V S K A V P K D G G T N L V Q	700
2101	GTAGGTAAAGGTATAGCTTCTTCAGTTATAAACAAAATGAAAGATTTATTAGATATG	2157
701	V G K G I A S S V I N K M K D L L D M	719

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 7A

SEQ ID No 6. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 171448, PCR type 12, with translation. The putative secretory signal cleavage site ( $\Delta$ ) and site of cleavage to form the two mature SLPs ( $\diamond$ ) are indicated.

```
1  ATGAATAAGAAAAATATAGCAATAGCTATGTCAGGTTTAAACAGTTTCTCGGCTGCT  60
   -----+-----+-----+-----+-----+
1  M N K K N I A I A M S G L T V L A S A A  20

61  CCTGTTTTGCTGCAACTACTGGAACACAAGGTTATACTGTAGTTAAAAACGACTGGAAA  120
   -----+-----+-----+-----+-----+
21  P V F A A T T G T Q G Y T V V K N D W K  40
      Δ

121  AAAGCAGTAAACAATTACAAGATGGACTAAAAGATAATAGTATAGGAAAGATAACTGTA  180
   -----+-----+-----+-----+-----+
41  K A V K Q L Q D G L K D N S I G K I T V  60

181  TCTTTTAATGATGGGGTTGTGGGTGAAGTAGCTCCTAAAAGTGCTAATAAGAAAGCGGAC  240
   -----+-----+-----+-----+-----+
61  S F N D G V V G E V A P K S A N K K A D  80

241  AGAGATGCTGCAGCTGAGAAGTTATATAATCTTGTTAACTCAATTAGATAAATTAGGT  300
   -----+-----+-----+-----+-----+
81  R D A A A E K L Y N L V N T Q L D K L G  100

301  GATGGAGATTATGTTGATTTTTCTGTAGATTATAATTTAGAAAACAAAATAACTAAT  360
   -----+-----+-----+-----+-----+
101  D G D Y V D F S V D Y N L E N K I I T N  120

361  CAAGCAGATGCAGAAGCAATTGTTACAAAGTTAAATTCATTATGAGAAAACCTTATT  420
   -----+-----+-----+-----+-----+
121  Q A D A E A I V T K L N S L N E K T L I  140

421  GATATAGCAACTAAAGATACTTTTGAATGGTTAGTAAAACACAAGATAGTGGAGGTAAA  480
   -----+-----+-----+-----+-----+
141  D I A T K D T F G M V S K T Q D S G G K  160

481  AATGTTGCTGCAACAAAGGCACTTAAAGTTAAAGATGTTGCTACATTTGGTTTGAAGTCT  540
   -----+-----+-----+-----+-----+
161  N V A A T K A L K V K D V A T F G L K S  180

541  GGTGGAAGCGAAGATACTGGATATGTTGTTGAAATGAAAGCAGGAGCTGTAGAGGATAAG  600
   -----+-----+-----+-----+-----+
181  G G S E D T G Y V V E M K A G A V E D K  200

601  TATGGTAAAGTTGGAGATAGTACGGCAGGTATTGCAATAAATCTTCCTAGTACTGGACTT  660
   -----+-----+-----+-----+-----+
201  Y G K V G D S T A G I A I N L P S T G L  220
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 7B

661	GAATATGCAGGTAAAGGAACAACAATTGATTTTAATAAAACTTTAAAAGTTGATGTAACA	720
221	E Y A G K G T T I D F N K T L K V D V T	240
721	GGTGGTTCAACACCTAGTGCTGTAGCTGTAAGTGGTTTGTAACTAAAGATGATACTGAT	780
241	G G S T P S A V A V S G F V T K D D T D	260
781	TTAGCAAAATCAGGTACTATAAATGTAAGAGTTATAAATGCAAAAGAAGAATCAATTGAT	840
261	L A K S G T I N V R V I N A K E E S I D	280
841	ATAGATGCAAGCTCATATACATCAGCTGAAAATTTAGCTAAAAGATATGTATTTGATCCA	900
281	I D A S S Y T S A E N L A K R Y V F D P	300
901	GATGAAATTTCTGAAGCATATAAGGCAATAGTAGCATTACAAAATGATGGTATAGAGTCT	960
301	D E I S E A Y K A I V A L Q N D G I E S	320
961	AATTTAGTTCAGTTAGTTAATGGAAAATATCAAGTGATTTTTATCCAGAAGGTAAAAGA	1020
321	N L V Q L V N G K Y Q V I F Y P E G K R	340
1021	TTAGAAACTAAATCAGCAAAATGATACAATAGCTAGTCAAGATACACCAGCTAAAGTAGTT	1080
341	L E T K S A N D T I A S Q D T P A K V V	360
1081	ATAAAAGCTAATAAATTAAAAGATTTAAAAGATTATGTAGATGATTTAAAAACATATAAT	1140
361	I K A N K L K D L K D Y V D D L K T Y N	380
1141	AATACTTATTCAAATGTTGTAACAGTAGCAGGAGAAGATAGAATAGAACTGCTATAGAA	1200
381	N T Y S N V V T V A G E D R I E T A I E	400
1201	TTAAGTAGTAAATATTATAATTCTGATGATAAAAATGCAATAACTGATAAAGCAGTTAAT	1260
401	L S S K Y Y N S D D K N A I T D K A V N	420
1261	GATATAGTATTAGTTGGATCTACATCTATAGTTGATGGTCTTGTGTCATCACCATTAGCT	1320
421	D I V L V G S T S I V D G L V A S P L A	440
1321	TCAGAAAAACAGCTCCATTATTATTAGCTTCAAAAGATAAATTAGATTTCATCAGTAAAA	1380
441	S E K T A P L L L A S K D K L D S S V K	460
1381	TCTGAAATAAAGAGAGTTATGAACTTAAAGAGTGACACTGGTATAAATACTTCTAAAAA	1440
461	S E I K R V M N L K S D T G I N T S K K	480

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 7C

1441	GTTTATTTAGCTGGTGGAGTTAATTCTATATCTAAAGATGTAGAAAATGAATTGAAAAAC	1500
481	V Y L A G G V N S I S K D V E N E L K N	500
1501	ATGGGTCTTAAAGTTACTAGATTATCAGGAGAAGACAGATACGAAACTTCTTTAGCAATA	1560
501	M G L K V T R L S G E D R Y E T S L A I	520
1561	GCTGATGAAATAGGTCTTGATAATGATAAAGCATTGTAGTTGGTGGTACTGGATTAGCA	1620
521	A D E I G L D N D K A F V V G G T G L A	540
1621	GATGCTATGAGTATAGCTCCAGTTGCTTCTCAACTTAAAGATGGAGATGCTACTCCAATA	1680
541	D A M S I A P V A S Q L K D G D A T P I	560
1681	GTAGTTGTAGATGGAAAAGCAAAAGAAATAAGTGATGATGCTAAGAGTTTCTTAGGAACT	1740
561	V V V D G K A K E I S D D A K S F L G T	580
1741	TCTGATGTTGATATAATAGGTGGAAAAATAGCGTATCTAAAGAGATTGAAGAGTCAATA	1800
581	S D V D I I G G K N S V S K E I E E S I	600
1801	GATAGTGCAACTGGAAAACTCCAGATAGAATAAGTGGAGATGATAGACAAGCAACTAAT	1860
601	D S A T G K T P D R I S G D D R Q A T N	620
1861	GCTGAAGTTTTAAAAGAAGATGATTATTTACAGATGGTGAAGTTGTGAATTACTTTGTT	1920
621	A E V L K E D D Y F T D G E V V N Y F V	640
1921	GCAAAAGATGGTTCTACTAAGAAGATCAATTAGTAGATGCCTTAGCAGCAGCACCATA	1980
641	A K D G S T K E D Q L V D A L A A A P I	660
1981	GCAGGTAGATTTAAGGAGTCTCCAGCTCCAATCATACTAGCTACTGATACTTTATCTTCT	2040
661	A G R F K E S P A P I I L A T D T L S S	680
2041	GACCAAAATGTAGCTGTAAGTAAAGCAGTTCCTAAAGATGGTGGAACTAACTTAGTTCAA	2100
681	D Q N V A V S K A V P K D G G T N L V Q	700
2101	GTAGGTAAAGGTATAGCTTCTTCAGTTATAAACAATGAAAGATTTATTAGATATG	2157
701	V G K G I A S S V I N K M K D L L D M	719

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 8A

SEQ ID No. 7. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 171862, PCR type 17, with translation. The putative secretory signal cleavage site ( $\Delta$ ) and site of cleavage to form the two mature SLPs ( $\diamond$ ) are indicated.

```
1 ATGAATAAGAAAACCTAGCAATGGCTATGGCAGCAGTTACTGTTGTGGGTTCTGCAGCG 60
-----+-----+-----+-----+-----+
1 M N K K N L A M A M A A V T V V G S A A 20

61 CCAATATTTGCAGATAGTACTACGCCAGGTTATACTGTAGTGAAAAATGATTGGAAAAAA 120
-----+-----+-----+-----+-----+
21 P I F A D S T T P G Y T V V K N D W K K 40
      Δ

121 GCAGTAAACAATTACAAGATGGGTTGAAAAATAAACTATATCAACAATAAAGGTGTCT 180
-----+-----+-----+-----+-----+
41 A V K Q L Q D G L K N K T I S T I K V S 60

181 TTTAATGGAAACTCTGTTGGAGAAGTTACACCAGCCAGTTCTGGAGCAAAAAAGCAGAT 240
-----+-----+-----+-----+-----+
61 F N G N S V G E V T P A S S G A K K A D 80

241 AGAGATGCTGCAGCTGAAAAGTTATATAATTTAGTAAATACACAATTAGATAAACTAGGT 300
-----+-----+-----+-----+-----+
81 R D A A A E K L Y N L V N T Q L D K L G 100

301 GATGGAGATTACGTTGACTTTGAAGTAACTTATAATTTAGCTACTCAAATAATTACAAAA 360
-----+-----+-----+-----+-----+
101 D G D Y V D F E V T Y N L A T Q I I T K 120

361 GCAGAAGCAGAGGCAGTTCTTACAAAATTACAACAATATAATGATAAAGTACTTATAAAT 420
-----+-----+-----+-----+-----+
121 A E A E A V L T K L Q Q Y N D K V L I N 140

421 TCTGCAACAGATACAGTAAAAGGTATGGTATCTGATACACAAGTTGATAGCAAAAATGTT 480
-----+-----+-----+-----+-----+
141 S A T D T V K G M V S D T Q V D S K N V 160

481 GCAGCTAACCCTAAAGTTAGTGATATGTATACAATACCATCTGCTATTACTGGAAGT 540
-----+-----+-----+-----+-----+
161 A A N P L K V S D M Y T I P S A I T G S 180

541 GATGATTCTGGGTATAGTATTGCTAAACCAACAGAAAAGACTACAGTTTATTGTATGGT 600
-----+-----+-----+-----+-----+
181 D D S G Y S I A K P T E K T T S L L Y G 200

601 ACGGTTGGTGATGCAACTGCAGGTAAAGCAATAACAGTAGATACAGCTTCAATGAAGCT 660
-----+-----+-----+-----+-----+
201 T V G D A T A G K A I T V D T A S N E A 220
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 8B

661	TTTGCTGGAAATGGAAAGGTTATTGACTACAATAAATCATTCAAAGCAACTGTACAAGGA	720
221	F A G N G K V I D Y N K S F K A T V Q G	240
721	GATGGAACAGTTAAGACAAGCGGGTTGTTACTTAAAGATGCAAGTGATATGGCTGCAACA	780
241	D G T V K T S G V V L K D A S D M A A T	260
781	GGTACTATAAAAGTTAGAGTTACAAGTGCAAAAGAAGAATCTATTGATGTGGATTCAAGT	840
261	G T I K V R V T S A K E E S I D V D S S	280
841	TCATATATTAGTGCTGAAAATTTAGCTAAAAATATGTATTTAATCCTAAAGAGGTTTCT	900
281	S Y I S A E N L A K K Y V F N P K E V S	300
901	GAAGCTTATAATGCAATAGTTGCATTACAAAATGATGGAATAGAATCTGATTTAGTACAA	960
301	E A Y N A I V A L Q N D G I E S D L V Q	320
961	TTAGTTAATGGAAAATATCAAGTTATTTCTATCCAGAAGGAAAAAGATTAGAACTAAA	1020
321	L V N G K Y Q V I F Y P E G K R L E T K	340
1021	TCTGCAGATATAATAGCTGATGCAGATAGTCCAGCTAAAATAACTATAAAAGCTAATAAA	1080
341	S A D I I A D A D S P A K I T I K A N K	360
1081	TTAAAAGATTTAAAAGATTATGTAGATGATTTAAAAACATACAATAATACTTACTCAAAT	1140
361	L K D L K D Y V D D L K T Y N N T Y S N	380
1141	GTTGTAACAGTAGCAGGAGAAGATAGAATAGAAACTGCTATAGAATTAAGTAGTAAATAT	1200
381	V V T V A G E D R I E T A I E L S S K Y	400
1201	TATAATTCTGATGATAAAAATGCAATAACTGATGATGCAGTTAATAATATAGTATTAGTT	1260
401	Y N S D D K N A I T D D A V N N I V L V	420
1261	GGATCTACATCTATAGTTGATGGTCTTGTTCATCACCATTAGCTTCAGAAAAACAGCT	1320
421	G S T S I V D G L V A S P L A S E K T A	440
1321	CCATTATTATTAACCTCAAAGATAAATTAGATTCATCAGTAAAATCTGAGATAAAAAGA	1380
441	P L L L T S K D K L D S S V K S E I K R	460
1381	GTTATGAACCTAAAGAGTGATACTGGTATAAATACTTCTAAAAAGTTTATTAGCTGGT	1440
461	V M N L K S D T G I N T S K K V Y L A G	480

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 8C

```
1441 GGAGTTAATTCATATCTAAAGATGTAGAAGATGAATTGAAAAATATGGGCCTTAAAGTT 1500
-----+-----+-----+-----+-----+-----+
481 G V N S I S K D V E D E L K N M G L K V 500

1501 ACTAGATTATCAGGAGAAGACAGATACGAACTTCTTTAGCAATAGCTGATGAAATAGGT 1560
-----+-----+-----+-----+-----+-----+
501 T R L S G E D R Y E T S L A I A D E I G 520

1561 CTTGATAATGATAAAGCATTGTGTAGTTGGTGGTACTGGATTGGCAGATGCTATGAGTATA 1620
-----+-----+-----+-----+-----+-----+
521 L D N D K A F V V G G T G L A D A M S I 540

1621 GCTCCAGTTGCTTCTCAACTTAAAGATGGAGATGCTACTCCAATAGTAGTTGTAGATGGA 1680
-----+-----+-----+-----+-----+-----+
541 A P V A S Q L K D G D A T P I V V V D G 560

1681 AAAGCAAAGAAATAAGTGATGATGCTAAGAGTTTCTTAGGAACTTCTGATGTTGATATA 1740
-----+-----+-----+-----+-----+-----+
561 K A K E I S D D A K S F L G T S D V D I 580

1741 ATAGGTGGAAAAATAGCGTATCTAAAGAGATTGAAGAGTCAATAGATAGTGCAACTGGA 1800
-----+-----+-----+-----+-----+-----+
581 I G G K N S V S K E I E E S I D S A T G 600

1801 AAAACTCCAGATAGAATAAGTGGAGATGACAGACAAGCAACTAATGCTGAAGTTTAAAA 1860
-----+-----+-----+-----+-----+-----+
601 K T P D R I S G D D R Q A T N A E V L K 620

1861 GAAGATGATTATTTCAAAGATGGTGAAGTTGTGAATTACTTTGTGCAAAAGATGGTTCT 1920
-----+-----+-----+-----+-----+-----+
621 E D D Y F K D G E V V N Y F V A K D G S 640

1921 ACTAAGAAGATCAATTAGTAGATGCATTAGCAGCAGCACCAATAGCAGGTAGATTTAAG 1980
-----+-----+-----+-----+-----+-----+
641 T K E D Q L V D A L A A A P I A G R F K 660

1981 GAGTCTCCAGCTCCAATCATACTAGCTACTGATACTTTATCTTCTGACCAAATGTAGCT 2040
-----+-----+-----+-----+-----+-----+
661 E S P A P I I L A T D T L S S D Q N V A 680

2041 GTAAGTAAAGCAGTTCCTAAAGATGGTGGAACTAACTTAGTTCAAGTAGGTAAAGGTATA 2100
-----+-----+-----+-----+-----+-----+
681 V S K A V P K D G G T N L V Q V G K G I 700

2101 GCTTCTTCAGTTATAAACAAAATGAAAGATTATTAGATATGTAA 2145
-----+-----+-----+-----+-----+
701 A S S V I N K M K D L L D M * 715
```



Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 9A

SEQ ID No 8. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 173644, PCR type 31, with translation. The putative secretory signal cleavage site ( $\Delta$ ) and site of cleavage to form the two mature SLPs ( $\diamond$ ) are indicated.

```
1 ATGAATAAGAAGGATATAGCAATAGCTATGTCAGGATTAACAGTATTAGCTTCTGCAGCA 60
  +-----+-----+-----+-----+
1 M N K K D I A I A M S G L T V L A S A A 20

61 CCTGTATTGCTGCTAGTAGTTTACAGCAGATTATAATTATACTGTAGTGCAAGGAAAA 120
  +-----+-----+-----+-----+
21 P V F A A S S F T A D Y N Y T V V Q G K 40
    Δ

121 TATCAAAAAGTTATAACTGGATTACAAGATGGTTTAAAAAATGGAAAAATAACAAATATT 180
  +-----+-----+-----+-----+
41 Y Q K V I T G L Q D G L K N G K I T N I 60

181 GATGTAATATTTGATGGAAGTTCAATTGCTGAGGTAGTGCCAGGTCTGTATGCTGCAGCT 240
  +-----+-----+-----+-----+
61 D V I F D G S S I G E V V P G S D A A A 80

241 GCAGCTACTAAATTAAAAAGTTTAGTTGATGATAAGTTAGATAACTTAGGTGATGGAAAA 300
  +-----+-----+-----+-----+
81 A A T K L K S L V D D K L D N L G D G K 100

301 TACGTTCAATTTAATGTTACTTATACTACTAAATCTATAATACTAAAGCAGAATTAAAA 360
  +-----+-----+-----+-----+
101 Y V Q F N V T Y T T K S I I T K A E L K 120

361 AATTATTATAATCAATTAGAAAGTAGTAAAGATAGAATACTTATAGGAAATGAACCTCAA 420
  +-----+-----+-----+-----+
121 N Y Y N Q L E S S K D R I L I G N E P Q 140

421 GATACAGGAAGTAAGGTCTTATAAAGCTGATACTGATGGTACTACTGCTGTTGCAGCA 480
  +-----+-----+-----+-----+
141 D T G T K G L I K A D T D G T T A V A A 160

481 GCTGCACCATTTGAAATTATCAGATATATTTACGTTTAGTTATGATGAAGTAACAGGTGTA 540
  +-----+-----+-----+-----+
161 A A P L K L S D I F T F S Y D E V T G V 180

541 CTTAAAGCAGAACCAACAAGTAAGTAAGCGCTGGTAAAGTTCAAGGTCTAAATATGGA 600
  +-----+-----+-----+-----+
181 L K A E P T S K V S A G K V Q G L K Y G 200

601 AATACAGGAGCAACTAACTATACTTCTGGAGCTGAAATATCTGTTCTACTACAGGCTTA 660
  +-----+-----+-----+-----+
201 N T G A T N Y T S G A E I S V P T T G L 220
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 9B

```
661 ACATTAAGTCTGATACAACTGCAACAACAGATGTAAATATTTCTGATGTTATGAGTGCA 720
-----+-----+-----+-----+-----+
221 T L T A D T T A T T D V N I S D V M S A 240

721 TTAAATTTAATGGTACTGATACGATTAGTGGATTCCCAGCTGGTTCATCAGCTTCTACT 780
-----+-----+-----+-----+-----+
241 F K F N G T D T I S G F P A G S S A S T 260

781 CTTAGAGCAAGTATAAAAGTAATAAATGCAAAAGAAGAATCTATAGATGTTGATTCAAGT 840
-----+-----+-----+-----+-----+
261 L R A S I K V I N A K E E S I D V D S S 280

841 TCACATAGAAGCTGAAGATTTAGCTGAAAAATATGTATTTAAACCAGAAGATGTGAAT 900
-----+-----+-----+-----+-----+
281 S H R T A E D L A E K Y V F K P E D V N 300

901 AAACTTATGAGGCACTGACTGATTATATAAAGAAGGTATAACAAGTAATCTTATCACT 960
-----+-----+-----+-----+-----+
301 K T Y E A L T D L Y K E G I T S N L I T 320

961 CAAGATGGTGGAAATATCAAGTTGTTTATTTGCTCAAGGAAAGAGATTAAGTACTAAA 1020
-----+-----+-----+-----+-----+
321 Q D G G K Y Q V V L F A Q G K R L T T K 340

1021 GGAGCAACTGGAACCTTAGCAGATGAAATCTCCTCTTAAAGTAACAATAAAGCAGAT 1080
-----+-----+-----+-----+-----+
341 G A T G T L A D E N S P L K V T I K A D 360
♦

1081 AAAGTAAAGACTTAAAGATTATGTTGAAGATTTAAAAATGCTAACAATGGATATTCA 1140
-----+-----+-----+-----+-----+
361 K V K D L K D Y V E D L K N A N N G Y S 380

1141 AATTCTGTTGTTGTAGCAGGTGAAGATAGAATAGAAACAGCAATAGAGTTAAGTAGCAAA 1200
-----+-----+-----+-----+-----+
381 N S V V V A G E D R I E T A I E L S S K 400

1201 TACTATAACTCTGATGATGACAATGCAATAACTAAAGATCCAGTTAACAATGTTGTTTAA 1260
-----+-----+-----+-----+-----+
401 Y Y N S D D D N A I T K D P V N N V V L 420

1261 GTTGGTTCTCAAGCTGTAGTTGATGGGCTTGTAGCTTACCTTTAGCATCTGAAAAAGA 1320
-----+-----+-----+-----+-----+
421 V G S Q A V V D G L V A S P L A S E K R 440

1321 GCTCCTTTACTATTAACTTCAGCAGGAAATTAGATTCAAGTGTTAAAGCTGAGTTGAAA 1380
-----+-----+-----+-----+-----+
441 A P L L L T S A G K L D S S V K A E L K 460

1381 AGAGTAATGGATTTAAATCTACAACAGGTGTAAATCTTCTAAAAAGTTTACTTAGCT 1440
-----+-----+-----+-----+-----+
461 R V M D L K S T T G V N T S K K V Y L A 480
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 9C

1441	GGTGGAGTAAACTCTATATCTAAAGATGTAGAAAATGAATTAAAAGATATGGGACTTAAA	1500
481	G G V N S I S K D V E N E L K D M G L K	500
1501	GTTACAAGATTATCAGGAGATGATAGATATGAAACTTCTTTAGCTATAGCTGATGAAATA	1560
501	V T R L S G D D R Y E T S L A I A D E I	520
1561	GGTCTTGATAATGATAAAGCTTTTGTAGTTGGAGGAACAGGATTAGCGGATGCTATGAGT	1620
521	G L D N D K A F V V G G T G L A D A M S	540
1621	ATAGCTCCAGTTGCTTCTCAATTAAGAAACTCAAATGGAGAACTTGACTTAAAAGGTGAT	1680
541	I A P V A S Q L R N S N G E L D L K G D	560
1681	GCAACTCCAATAGTAGTTGTTGATGGAAAAGCTAAAGATATAAATTCTGAAGTAAAAGAT	1740
561	A T P I V V V D G K A K D I N S E V K D	580
1741	TTCTTAGATGATTACAAGTTGATATAATAGGTGGTGTAATAGTGTCTTCTAAAGAAGTA	1800
581	F L D D S Q V D I I G G V N S V S K E V	600
1801	ATGGAAGCAATAGATGATGCTACTGGAAAATCACCTGAGAGATATAGTGGAGAAGATAGA	1860
601	M E A I D D A T G K S P E R Y S G E D R	620
1861	CAAGCAACAAATGCTAAAGTTATAAAGAAGATGATTCTTTAAAAATGGAGAAGTTACA	1920
621	Q A T N A K V I K E D D F F K N G E V T	640
1921	AACTTCTTTGTAGCTAAAGATGGTTCAACTAAAGAAGATCAATTAGTAGATGCTTTAGCA	1980
641	N F F V A K D G S T K E D Q L V D A L A	660
1981	GGTGCTGCAATTGCTGGTAACTTTGGTGTAACAGTAGATAATGAAGGAAAACCTACAGTT	2040
661	G A A I A G N F G V T V D N E G K P T V	680
2041	GCTGATAAAAAAGCTTCTCCAGCACCAATTGTTTTAGCAACAGATTCTTTATCTTCTGAT	2100
681	A D K K A S P A P I V L A T D S L S S D	700
2101	CAAAATGTAGCTATAAGTAAAGCTGTAAATGATGACGCTAATACTAAGAATCTAGTTCAA	2160
701	Q N V A I S K A V N D D A N T K N L V Q	720

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 9D

2161	GTTGGTAAAGGTATAGCTACTTCAGTTGTAAGTAAATAAAAGATTATTAGATATG	2217
-----+-----+-----+-----+-----		
721	V G K G I A T S V V S K I K D L L D M	739

Appl. No: 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 10A

SEQ ID No 9. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 170444, PCR type 46, with translation. The putative secretory signal cleavage site ( $\Delta$ ) and site of cleavage to form the two mature SLPs ( $\diamond$ ) are indicated.

```
1 ATGAATAAGAAAAATATAGCAATAGCTATGTCAGGTTTAAACAGTTTCTCGGCTGCT 60
  -----+-----+-----+-----+-----+
1 M N K K N I A I A M S G L T V L A S A A 20

61 CCTGTTTTTGCTGCAACTACTGGAACACAAGGTTTACTGTAGTTAAAAACGACTGGAAA 120
  -----+-----+-----+-----+-----+
21 P V F A A T T G T Q G Y T V V K N D W K 40
    Δ

121 AAAGCAGTAAAACAATTACAAGATGGACTAAAAGATAATAGTATAGGAAAGATAACTGTA 180
  -----+-----+-----+-----+-----+
41 K A V K Q L Q D G L K D N S I G K I T V 60

181 TCTTTTAAATGATGGGTTGTGGGTGAAGTAGCTCCTAAAAGTGCTAATAAGAAAGCGGAC 240
  -----+-----+-----+-----+-----+
61 S F N D G V V G E V A P K S A N K K A D 80

241 AGAGATGCTGCAGCTGAGAAGTTATATAATCTTGTTAACTCAATTAGATAAATTAGGT 300
  -----+-----+-----+-----+-----+
81 R D A A A E K L Y N L V N T Q L D K L G 100

301 GATGGAGATTATGTTGATTTTCTGTAGATTATAATTTAGAAAAAAAATAATACTAAT 360
  -----+-----+-----+-----+-----+
101 D G D Y V D F S V D Y N L E K K I I T N 120

361 CAAGCAGATGCAGAAGCAATTGTTACAAAGTTAAATTCACCTAATGAGAAAACCTCTTATT 420
  -----+-----+-----+-----+-----+
121 Q A D A E A I V T K L N S L N E K T L I 140

421 GATATAGCAACTAAAGATACTTTTGAATGGTTAGTAAAACACAAGATAGTGAAGGTAAA 480
  -----+-----+-----+-----+-----+
141 D I A T K D T F G M V S K T Q D S E G K 160

481 AATGTTGCTGCAACAAAGGCACTTAAAGTTAAAGATGTTGCTACATTTGGTTTGAAGTCT 540
  -----+-----+-----+-----+-----+
161 N V A A T K A L K V K D V A T F G L K S 180

541 GGTGGAAGCGAAGATACTGGATATGTTATTGAAATGAAAGCAGGAGCTGTAGAGGATAAG 600
  -----+-----+-----+-----+-----+
181 G G S E D T G Y V I E M K A G A V E D K 200

601 TATGGTAAAGTTGGAGATAGTACGGCAGGTATTGCAATAAATCTTCCTAGTACTGGACTT 660
  -----+-----+-----+-----+-----+
201 Y G K V G D S T A G I A I N L P S T G L 220
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 10B

```
661 GAATATGCAGGTAAAGGAACAACAATTGATTTTAATAAACTTTAAAAGTTGATGTAACA 720
-----+-----+-----+-----+
221 E Y A G K G T T I D F N K T L K V D V T 240

721 GGTGGTTCAACACCTAGTGCTGTAGCTGTAAGTGGTTTTGTAAGTAAAGATGATACTGAT 780
-----+-----+-----+-----+
241 G G S T P S A V A V S G F V T K D D T D 260

781 TTAGCAAAATCAGGTACTATAAATGTAAGAGTTATAAATGCAAAAGAAGAATCAATTGAT 840
-----+-----+-----+-----+
261 L A K S G T I N V R V I N A K E E S I D 280

841 ATAGATGCAAGCTCATATACATCAGCTGAAAATTAGCTAAAAGACATGTATTTGATCCA 900
-----+-----+-----+-----+
281 I D A S S Y T S A E N L A K R H V F D P 300

901 GATGAAATTTCTGAAGCATATAAGGCAATAGTAGCATTACAAAATGATGGTATAGAGTCT 960
-----+-----+-----+-----+
301 D E I S E A Y K A I V A L Q N D G I E S 320

961 AATTTAGTTCAGTTAGTTAATGGAAAATATCAAGTGATTTTTTATCCAGAAGGTAAAAGA 1020
-----+-----+-----+-----+
321 N L V Q L V N G K Y Q V I F Y P E G K R 340

1021 TTAGAACTAAATCAGCAAAATGATACAATAGCTAGTCAAGATACACCAGCTAAAGTAGTT 1080
-----+-----+-----+-----+
341 L E T K S A N D T I A S Q D T P A K V V 360

1081 ATAAAAGCTAATAAATTTAAAGATTATAAAGATTATGTAGATGATTTAAAAACATATAAT 1140
-----+-----+-----+-----+
361 I K A N K L K D L K D Y V D D L K T Y N 380

1141 AATACTTATTCAAATGTTGTAACAGTAGCAGGAGAAGATAGAATAGAACTGCTATAGAA 1200
-----+-----+-----+-----+
381 N T Y S N V V T V A G E D R I E T A I E 400

1201 TTAAGTAGTAAATATTATAATTCTGATGATAAAAATGCAATAACTGATAAAGCAGTTAAT 1260
-----+-----+-----+-----+
401 L S S K Y Y N S D D K N A I T D K A V N 420

1261 GATATAGTATTAGTTGGATCTACATCTATAGTTGATGGTCTTGTTGCATCACCATTAGCT 1320
-----+-----+-----+-----+
421 D I V L V G S T S I V D G L V A S P L A 440

1321 TCAGAAAAACAGCTCCATTATTATTAACCTTCAAAGATAAATTAGATTCATCAGTAAAA 1380
-----+-----+-----+-----+
441 S E K T A P L L L T S K D K L D S S V K 460

1381 TCTGAAATAAAGAGAGTTATGAACTTAAAGAGTGACACTGGTATAAATACTTCTAAAAAA 1440
-----+-----+-----+-----+
461 S E I K R V M N L K S D T G I N T S K K 480
```

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 10C

1441	GTTTATTTAGCTGGTGGAGTTAATCTATATCTAAAGATGTAGAAATGAATTGAAAAAC	1500
481	V Y L A G G V N S I S K D V E N E L K N	500
1501	ATGGGTCTTAAAGTTACTAGATTATCAGGAGAAGACAGATACGAACTTCTTTAGCAATA	1560
501	M G L K V T R L S G E D R Y E T S L A I	520
1561	GCTGATGAATAGGTCTTGATAATGATAAAGCATTGTAGTTGGTGGTACTGGATTAGCA	1620
521	A D E I G L D N D K A F V V G G T G L A	540
1621	GATGCTATGAGTATAGCTCCAGTTGCTTCTCAACTTAAAGATGGAGATGCTACTCCAATA	1680
541	D A M S I A P V A S Q L K D G D A T P I	560
1681	GTAGTTGTAGATGGAAAAGCAAAAGAAATAAGTGATGATGCTAAGAGTTTCTTAGGAACT	1740
561	V V V D G K A K E I S D D A K S F L G T	580
1741	TCTGATGTTGATATAATAGGTGGAAAAATAGCGTATCTAAAGAGATTGAAGAGTCAATA	1800
581	S D V D I I G G K N S V S K E I E E S I	600
1801	GATAGTGCAACTGGAAAACTCCAGATAGAATAAGTGGAGATGATAGACAAGCAACTAAT	1860
601	D S A T G K T P D R I S G D D R Q A T N	620
1861	GCTGAAGTTTAAAAGAAGATGATTATTTACAGATGGTGAAGTTGTGAATTACTTTGTT	1920
621	A E V L K E D D Y F T D G E V V N Y F V	640
1921	GCAAAAGATGGTTCTACTAAAGAAGATCAATTAGTAGATGCCTTAGCAGCAGCACCATA	1980
641	A K D G S T K E D Q L V D A L A A A P I	660
1981	GCAGGTAGATTTAAGGAGTCTCCAGCTCCAATCATACTAGCTACTGATACTTTATCTTCT	2040
661	A G R F K E S P A P I I L A T D T L S S	680
2041	GACCAAATGTAGCTGTAAGTAAAGCAGTTCCTAAAGATGGTGGAACTAACTTAGTTCAA	2100
681	D Q N V A V S K A V P K D G G T N L V Q	700
2101	GTAGGTAAAGGTATAGCTTCTTCAGTTATAAACAAATGAAAGATTTATTAGATATG	2157
701	V G K G I A S S V I N K M K D L L D M	719

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 11A

SEQ ID No 10. Nucleotide sequence of *slpA* from *Clostridium difficile* strain 170426, PCR type 92, with translation. The putative secretory signal cleavage site ( $\Delta$ ) and site of cleavage to form the two mature SLPs ( $\diamond$ ) are indicated.

```
1  ATGAATAAGAAAAATATAGCAATAGCTATGTCAGGTTTAAACAGTTTTAGCTTCGGCTGCT  60
   -----+-----+-----+-----+-----+
1  M N K K N I A I A M S G L T V L A S A A  20

61  CCTGTTTTTGCTGCAACTACTGGAACACAAGGTTATACTGTAGTTAAAAACGACTGGAAA  120
   -----+-----+-----+-----+-----+
21  P V F A A T T G T Q G Y T V V K N D W K  40
      Δ

121  AAAGCAGTAAACAATTACAGGATGGACTAAAGATAATAGTATAGGAAAGATAACTGTA  180
   -----+-----+-----+-----+-----+
41  K A V K Q L Q D G L K D N S I G K I T V  60

181  TCTTTTAATGATGGGGTTGTGGGTGAAGTAGCTCCTAAAAGTGCTAATAAGAAAGCGGAC  240
   -----+-----+-----+-----+-----+
61  S F N D G V V G E V A P K S A N K K A D  80

241  AGAGATGCTGCAGCTGAGAAGTTATATAATCTTGTTAACTCAATTAGATAAATTAGGT  300
   -----+-----+-----+-----+-----+
81  R D A A A E K L Y N L V N T Q L D K L G  100

301  GATGGAGATTATGTTGATTTTCTGTAGATTATAATTTAGAAAAAAAATAATAACTAAT  360
   -----+-----+-----+-----+-----+
101  D G D Y V D F S V D Y N L E K K I I T N  120

361  CAAGCAGATGCAGAAGCAATTGTTACAAAGTTAAATTCCTTAATGAGAAAACCTTATT  420
   -----+-----+-----+-----+-----+
121  Q A D A E A I V T K L N S L N E K T L I  140

421  GATATAGCAACTAAAGATACTTTTGAATGGTTAGTAAACACAAGATAGTGAAGGTAAA  480
   -----+-----+-----+-----+-----+
141  D I A T K D T F G M V S K T Q D S E G K  160

481  AATGTTGCTGCAACAAAGGCACTTAAAGTTAAAGATGTTGCTACATTGGTTTGAAGTCT  540
   -----+-----+-----+-----+-----+
161  N V A A T K A L K V K D V A T F G L K S  180

541  GGTGGAAGCGAAGATACTGGATATGTTGTTGAAATGAAAGCAGGAGCTGTAGAGGATAAG  600
   -----+-----+-----+-----+-----+
181  G G S E D T G Y V V E M K A G A V E D K  200

601  TATGGTAAAGTTGGAGATAGTACGGCAGGTATTGCAATAAATCTTCCTAGTACTGGACTT  660
   -----+-----+-----+-----+-----+
201  Y G K V G D S T A G I A I N L P S T G L  220
```



Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 11B

661 GAATATGCAGGTAAGGAACAACAATTGATTTTAATAAACTTTAAAGTTGATGTAACA 720  
-----+-----+-----+-----+  
221 E Y A G K G T T I D F N K T L K V D V T 240

721 GGTGGTTCAACACCTAGTGCTGTAGCTGTAAGTGGTTTTGTAAGTAAAGATGATACTGAT 780  
-----+-----+-----+-----+  
241 G G S T P S A V A V S G F V T K D D T D 260

781 TTAGCAAAATCAGGTACTATAAATGTAAGAGTTATAAATGCAAAAGAAGAATCAATTGAT 840  
-----+-----+-----+-----+  
261 L A K S G T I N V R V I N A K E E S I D 280

841 ATAGATGCAAGCTCATATACATCAGCTGAAAATTTAGCTAAAAGATATGTATTTGATCCA 900  
-----+-----+-----+-----+  
281 I D A S S Y T S A E N L A K R Y V F D P 300

901 GATGAAATTTCTGAAGCATATAAGGCAATAGTAGCATTACAAAATGATGGTATAGAGTCT 960  
-----+-----+-----+-----+  
301 D E I S E A Y K A I V A L Q N D G I E S 320

961 AATTTAGTTCAGTTAGTTAATGGAAAATATCAAGTGATTTTTATCCAGAAGGTAAAAGA 1020  
-----+-----+-----+-----+  
321 N L V Q L V N G K Y Q V I F Y P E G K R 340

1021 TTAGAAACTAAATCAGCAATGATACAATAGCTAGTCAAGATACACCAGCTAAAGTAGTT 1080  
-----+-----+-----+-----+  
341 L E T K S A N D T I A S Q D T P A K V V 360

1081 ATAAAAGCTAATAAATTAAGATTAAAGATTATGTAGATGATTTAAAAACATATAAT 1140  
-----+-----+-----+-----+  
361 I K A N K L K D L K D Y V D D L K T Y N 380

1141 AATACTTATTCAAATGTTGTAACAGTAGCAGGAGAAGATAGAATAGAAACTGCTATAGAA 1200  
-----+-----+-----+-----+  
381 N T Y S N V V T V A G E D R I E T A I E 400

1201 TTAAGTAGTAAATATTATAATTCTGATGATAAAATGCAATAACTGATAAAGCAGTTAAT 1260  
-----+-----+-----+-----+  
401 L S S K Y Y N S D D K N A I T D K A V N 420

1261 GATATAGTATTAGTTGGATCTACATCTATAGTTGATGGTCTTGTTCATCACCATTAGCT 1320  
-----+-----+-----+-----+  
421 D I V L V G S T S I V D G L V A S P L A 440

1321 TCAGAAAAACAGCTCCATTATTATTAAGTTCAAAAGATAAATTAGATTCATCAGTAAAA 1380  
-----+-----+-----+-----+  
441 S E K T A P L L L T S K D K L D S S V K 460

1381 TCTGAATAAAGAGAGTTATGAAGTAAAGAGTGACACTGGTATAAATACTTCTAAAAAA 1440  
-----+-----+-----+-----+  
461 S E I K R V M N L K S D T G I N T S K K 480

Appl. No. 10/068,870  
Amdt. Dated July 28, 2005  
Reply to Office Action of Apr. 28, 2005  
Replacement Sheet (New)

Fig. 11C

1441	GTATTATTAGCTGGTGGAGTTAATCTATATCTAAAGATGTAGAAAATGAATTGAAAAAC	1500
481	V Y L A G G V N S I S K D V E N E L K N	500
1501	ATGGGTCTTAAAGTTACTAGATTATCAGGAGAAGACAGATACGAAACTTCTTTAGCAATA	1560
501	M G L K V T R L S G E D R Y E T S L A I	520
1561	GCTGATGAAATAGGTCTTGATAATGATAAAGCATTGTAGTTGGTGGTACTGGATTAGCA	1620
521	A D E I G L D N D K A F V V G G T G L A	540
1621	GATGCTATGAGTATAGCTCCAGTTGCTTCTCAACTTAAAGATGGAGATGCTACTCCAATA	1680
541	D A M S I A P V A S Q L K D G D A T P I	560
1681	GTAGTTGTAGATGGAAAAGCAAAAGAAATAAGTGATGATGCTAAGAGTTTCTTAGGAAC	1740
561	V V V D G K A K E I S D D A K S F L G T	580
1741	TCTGATGTTGATATAATAGGTGGAAAAATAGCGTATCTAAAGAGATTGAAGAGTCAATA	1800
581	S D V D I I G G K N S V S K E I E E S I	600
1801	GATAGTGCAACTGGAAAAACTCCAGATAGAATAAGTGGAGATGATAGACAAGCAACTAAT	1860
601	D S A T G K T P D R I S G D D R Q A T N	620
1861	GCTGAAGTTTAAAAGAAGATGATTATTTACAGATGGTGAAGTTGTGAATTACTTTGTT	1920
621	A E V L K E D D Y F T D G E V V N Y F V	640
1921	GCAAAAGATGGTCTACTAAAGAAGATCAATTAGTAGATGCCTTAGCAGCAGCACCATA	1980
641	A K D G S T K E D Q L V D A L A A A P I	660
1981	GCAGGTAGATTTAAGGAGTCTCCAGCTCCAATCATACTAGCTACTGATACTTTATCTTCT	2040
661	A G R F K E S P A P I I L A T D T L S S	680
2041	GACCAAAATGTAGCTGTAAGTAAAGCAGTTCCTAAAGATGGTGGAACTAACTTAGTTCAA	2100
681	D Q N V A V S K A V P K D G G T N L V Q	700
2101	GTAGGTAAAGGTATAGCTTCTTCAGTTATAAACAAAATGAAAGATTTATTAGATATG	2157
701	V G K G I A S S V I N K M K D L L D M	719